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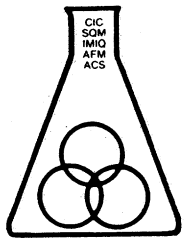

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Chapter 17

Cellulose Biosynthesis

The Terminal Complex Hypothesis and Its Relationship to Other Contemporary Research Topics

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Cellulose biosynthesis is a complex, sensitive, and not fully characterized process that occurs in organisms ranging from plants to bacteria to animals. Two fundamental approaches have been used to investigate cellulose biosynthesis; one structural and the other biochemical. The terminal complex hypothesis proposes that the cellulose synthesizing enzyme complex can be visualized with electron microscopy. Terminal complex is the name given to collections of plasma membrane particles thought to represent the cellulose synthase. While direct evidence is still not available to support this hypothesis, the amount of indirect supporting evidence has grown dramatically in the past few years. The relationship between terminal complexes, cellulose physical structure and the biochemical events of cellulose biosynthesis will be discussed.

Cellulose, a polysaccharide consisting of linear 1,4-β-D-anhydroglucopyranose chains laterally associated by hydrogen bonds, is the most abundant and commercially important plant cell wall polymer (1). Consequently, cellulose is also one of the most thoroughly investigated plant cell wall polymers. However, it is enigmatic in the sense that significant elements of cellulose physical structure and the mechanism of cellulose biosynthesis still are not well understood. Since these subjects have been reviewed recently (2-10), this review will update topics covered previously and provide a new analysis of selected topics of contemporary interest.

Cellulose Assembly

The terminal complex hypothesis proposes that structural manifestations of the cellulose synthase enzyme complex can be visualized with the freeze fracture specimen preparation technique for electron microscopy. These

structures, consisting of collections of intramembranous particles observed on the internal fracture faces of the plasma membrane, are frequently associated with the ends of cellulose microfibril impressions. Furthermore, since biochemical evidence has demonstrated that cellulose biosynthesis occurs at the plasma membrane and terminal complexes are located at the site of cellulose microfibril assembly, the hypothesis proposes that terminal complexes are the cellulose synthase protein complexes.

Since Roelofsen (11) and Preston (12) first provided the conceptual basis for the terminal complex hypothesis, these structures have been reported in a variety of organisms reviewed by Brown (6). The rosette/globule terminal complex consists of a collection of six particles arranged hexagonally on the protoplasmic face (PF) with a complementary globule on the exoplasmic face (EF) of the plasma membrane. Since the last review (6), rosette terminal complexes have been observed in the vascular plants *Lepidium sativum* L. (13) and *Zinnia elegans* (14), as well as in the green algae *Chara globularis* var. *capillacea* (15), *Nitella translucens* var. *axillaris* (16), and *Mougeotia* sp. (17,18). The linear terminal complex consists of particle rows (single, triple, or diagonal) and have been reported on the EF, PF, or both faces of the plasma membrane. Linear terminal complexes consisting of diagonal rows of PF intramembranous particles have been reported recently in *Vaucheria* of the Xanthophyceae (19). The remarkable dichotomy between the taxonomic distribution of the rosette/globule and linear types of terminal complexes continues to exist (18). Rosette/globule terminal complexes have been observed throughout the evolutionary spectrum of organisms from primitive plants such as green algae (Charophyceae) to advanced vascular plants. However, linear terminal complexes are only found in certain algal groups; the Chlorophyceae and Ulvophyceae classes in the green algae, the Xanthophyceae (yellow-green algae) and the Phaeophyceae (brown algae, *Pelvetia*, 20).

Direct microscopic evidence demonstrating that terminal complex particles are cellulose synthesizing enzymes is not currently available and will await the production of antibodies against cellulose synthase following its isolation and purification. However, the proposal that terminal complexes are part of the cellulose synthase complex is increasingly becoming accepted (2) due to the accumulation of indirect evidence supporting this hypothesis. Some of the most convincing data correlates high densities of terminal complexes with localized deposition of cellulose microfibrils during certain stages of plant cellular development. Rosette/globule terminal complex density values up to 191 per μm^2 were observed under the secondary cell wall thickenings of xylem tracheary elements of *Lepidium sativum* (13) and of *Zinnia elegans* (14). It has been known for quite some time that in tip-growing plant cells the density of rosette/globule terminal complexes increases dramatically at the tip (up to 48 rosettes per μm^2), where the most active cellulose microfibril deposition occurs (21,22).

The identification of terminal complexes in the Gram-negative bacterium *Acetobacter xylinum* now appears to be in doubt. Previously, a single linear row of particles observed on the outer lipopolysaccharide membrane

PF had been proposed as the terminal complex (23) and associated pores were reported on the outer membrane EF (24). Due to their proximity to the site of cellulose ribbon extrusion from the cell surface, these structures were assumed to be responsible for cellulose synthesis. A model was advanced in which cellulose synthase was localized on the outer membrane, which invoked adhesion sites between the outer and plasma membranes as a mechanism to explain the transfer of uridine-diphosphoryl-glucose (UDPG) from the cytoplasm to the cellulose synthases (25,26). However, when the outer and plasma membranes of *Acetobacter* were isolated separately by density-gradient centrifugation, the cellulose synthase activity was localized only in the plasma membrane fraction (27). Therefore, the linear structures observed on the *Acetobacter* outer membrane, while they may be associated in some manner with cellulose biosynthesis, are probably not the cellulose synthase terminal complexes. Since no ultrastructural evidence for adhesion sites between the outer and plasma membranes has been presented, a thorough investigation of the mechanism of β (1-4) glucan chain translocation from the cytoplasmic membrane to the outer membrane in *Acetobacter xylinum* is now in order.

Terminal Complex Structure and Phylogeny

Information derived from terminal complex structure has been used to probe phylogenetic relationships between cellulose producing organisms (6,17,18,26,28-30). As originally proposed (26), four characteristics of cellulose assembly (fixed vs. mobile sites of cellulose biosynthesis, linear vs. rosette terminal complexes, consolidated vs. unconsolidated terminal complexes, plasma membrane insertion of terminal complexes) were considered significant with regard to phylogenetic relationships. While most of these characteristics are still considered significant, their importance in determining phylogenetic relationships has been reinterpreted. Consequently, changes were made in the relative positions of organisms possessing terminal complexes in phylogenetic schemes which also reflect other ultrastructural and biochemical characteristics (18).

Fixed vs. mobile sites of cellulose biosynthesis. The phylogenetic utility of the fixed vs. mobile site characteristic of cellulose biosynthesis reflects basic structural differences between prokaryotic and eukaryotic organisms. In eukaryotic organisms, cellulose is produced from terminal complexes that move in the plane of the "fluid-mosaic" plasma membrane by the force generated from microfibril assembly (31), and deposit cellulose so that it envelops the cell. In contrast, most cellulose-producing prokaryotic organisms (including *Acetobacter, Achromobacter, Aerobacter, Agrobacterium, Alcaligenes, Azotobacter, Pseudomonas* and *Rhizobium*; 32) extrude cellulose as a ribbonlike extracellular product from a single fixed site on the cell surface. However, a cellulosic extracellular layer was reported in *Sarcina* (33). It would not be possible for the mobile site mechanism of cellulose biosynthesis to exist in prokaryotes due to the complication of cellulose extrusion through the peptidyl glycan cell wall and outer membrane.

Consolidation of rosette/globule terminal complexes. The strongly conserved nature of terminal complex morphology in certain eukaryotic taxonomic groups led to the reorganization of phylogenetic relationships, which were based on cellulose biosynthesis, along either a rosette terminal complex pathway or a linear terminal complex pathway (28). The significance of terminal complex consolidation is most obvious with zygnematalean algae in the rosette pathway. In this case, both solitary terminal complexes and terminal complex rows are associated with microfibril assembly during primary wall formation, whereas hexagonal arrays of terminal complexes are involved in secondary wall microfibril assembly. These examples represent three levels of terminal complex consolidation. First, the solitary rosette consists of six intramembranous particles consolidated in a pattern that contains six-fold rotational symmetry. Second- and third-order consolidation is observed in the linear translation of rosette/globule terminal complexes into rows or hexagonal arrays.

Terminal complex consolidation has also been reported in vascular plants as loosely aligned files of rosettes associated with secondary wall formation (13,14,34,35). Similar rosette files were also observed during primary wall formation in rapidly elongating regions of *Avena* coleoptiles (6,36). When coleoptiles were gravistimulated, terminal complex disaggregation occurred only on the lower coleoptile hemicylinder as evidenced by the observation of solitary globule terminal complexes (6,36). It was proposed that solitary terminal complexes produced microfibrils with less intermicrofibrillar hydrogen bonding than was present between microfibrils deposited by consolidated terminal complexes, allowing the lower hemicylinder to bend upward (36).

There appears to be a direct correlation between terminal complex length (linear consolidation) and the width of the microfibril produced. The best example of this correlation is in the deposition of secondary wall microfibrils in *Micrasterias* by hexagonal arrays of rosettes (37). In this example, the longest row of rosettes (up to 16 rosettes) located in the center of the array was associated with the widest microfibrils (up to 28.5 nm), while shorter rows were associated with narrower microfibrils. The relationship between the number of rosettes in a row and microfibril width is not proportional, however, since rows of 5 rosettes were associated with the deposition of 20 nm microfibrils in *Spirogyra* (38) and solitary rosettes were associated with 8 nm microfibrils in *Mougeotia* (17). The loosely associated files of rosettes involved in secondary wall formation in vascular plants have less linear order than the rosette rows found in zygnematalean hexagonal arrays. Correspondingly, the microfibril widths of the former were narrower than those in the latter. The consequence of the two types of secondary wall formation is that the zygnematalean secondary cell wall is more rigid than that typical of vascular plants (30), a characteristic that may be mutually advantageous for each organism in a functional sense.

Consolidation of linear terminal complexes. The correlation between linear consolidation of terminal complexes and microfibril width does not appear to be as consistent for linear terminal complexes, although this correlation

was previously reported (2,39). *Oocystis* and *Boergesenia* had the same average terminal complex length (510 nm), which was greater than that observed in *Valonia* (350 nm) and in *Vaucheria* (192 nm), while the *Oocystis, Valonia* and *Vaucheria* microfibril widths were similar (20 nm) but 10 nm less than that of *Boergesenia* (19,39,40). The structural development of the linear terminal complex has recently been reported during the regeneration of *Boergesenia* and *Valonia* protoplasts following wounding (41). In both genera terminal complex linear consolidation was reported to increase during primary wall formation, reaching a maximum length as secondary wall formation commenced. Similar results also were observed in *Boodlea* (Siphonocladales) during primary and secondary wall formation (42). Thus, linear terminal complex consolidation appears to be a manifestation of the stage of cell wall development rather than a significant factor in the determination of microfibril dimensions.

Plasma membrane insertion of terminal complexes. While the rosette/globule terminal complexes observed in the Zygnematales (*Micrasterias, Closterium, Spirogyra* and *Mougeotia*) were previously thought to be more transmembrane than those typical of vascular plants (26,37), the former terminal complexes are now considered to be more closely related to the latter than to the transmembrane linear terminal complexes characteristic of the Ulvophyceae (18). This statement does not imply disagreement with the observation that part of the rosette structure may be pulled away with the globule when the leaflets of the plasma membrane separate during the freeze fracture process. Rosette substructure appears to be a universal characteristic of globular terminal complexes, since it has been observed in vascular plants (30,43) and in the Zygnematales (37). It now appears that while the entire rosette/globule terminal complex spans the plasma membrane based on observations of complementary double replicas (37), neither the rosette nor the globule individually are transmembrane particles as suggested previously (37).

Herth (29) first reported that the rosette terminal complex was characteristic of those algae (Charophyceae) which represent the evolutionary line that gave rise to higher land plants. Several other taxonomic characteristics also are thought to support the proposal that the Charophyceae represents this phylogenetic line (44). Therefore, the insertion of the rosette/globule terminal complex in the plasma membrane does not appear to be phylogenetically significant, whereas this is the case for the linear terminal complex. Variation in linear terminal complex plasma membrane insertion exists in organisms representing distinctly different taxonomic groups. Only EF linear terminal complexes are observed in *Oocystis* (Chlorophyceae), while those in *Vaucheria* (Xanthophyceae) are observed only on the PF and those characteristic of *Valonia* and *Boergesenia* (Ulvophyceae) are found on both the EF and PF. The taxonomic groups represented by those organisms possessing linear terminal complexes are not considered to be closely related in a phylogenetic sense based on other ultrastructural and biochemical characteristics (44).

Cellulose Structure

Variation in the physical structure of cellulose has been observed according to its source and developmental stage (2,5). This variation, which includes differences in microfibril crystallographic orientation, degree of polymerization (DP), transverse crystalline dimensions (crystallite size), patterns of glucan chain hydrogen bonding and glucan chain polarity, has made the basic crystalline structure of cellulose difficult to determine. X-ray diffraction studies have identified several crystalline polymorphs of cellulose (45). Cellulose isolated from plants and bacteria typically occurs in the form of cellulose I (native cellulose). The cellulose II polymorph is formed from cellulose I by treatment with alkali (mercerization) or by precipitation from solution. A reversal in glucan chain polarity from parallel to antiparallel is thought to result from the conversion from cellulose I to II. While cellulose I and II are the most common polymorphs, other forms (cellulose III, IV and X) have been reported (45).

The cellulosic microfibrils of *Acetobacter*, and those present in the primary and secondary walls of vascular plants are twisted and show no preferred crystallographic orientation relative to the cell surface. However, flat crystallographically oriented microfibrils are produced by siphonocladalean algae (*Valonia, Boergesenia*; Ulvophyceae; 46; Roberts and Hotchkiss, unpublished results), cladophoralean algae (*Cladophora, Chaetomorpha*; Ulvophyceae; 47,48), zygnematalean algae (*Mougeotia*; Charophyceae; 18) and xanthophycean algae (*Vaucheria*; 19) in which the 6.0Å lattice plane of cellulose is typically parallel to the cell surface (uniplanar orientation, 1). The flat *Spirogyra* (Zygnematales) microfibrils appear to have unusual uniplanar orientation, since either the 3.9Å or the 5.4Å lattice planes have been reported to parallel the cell surface (49). Unusual uniplanar orientation also has been reported in *Oedogonium* (Chlorophyceae), in which the 5.4Å lattice plane was observed to parallel the cell surface (1,50). Differences in cellulose microfibril crystallographic orientation within the Zygnematales are thought to result from the presence (*Spirogyra*) or absence (*Mougeotia*) of secondary wall formation (18). In this regard, it will be interesting to see if other zygnematalean algae with secondary wall formation (i.e., *Micrasterias, Closterium*) possess the same unusual uniplanar orientation.

Further cellulosic structural variation is displayed by DP and crystallite size parameters. *Acetobacter* and vascular plant primary wall celluloses are low in DP (2,000-6,000), while siphonocladalean and vascular plant secondary wall celluloses are relatively high in DP (> 10,000) (2). During cotton fiber development, the cellulose IV polymorph is produced during primary wall formation, while in secondary walls, cellulose I is observed (51). The cellulose crystallite size is highest in ulvophycean and certain chlorophycean algae (114-169Å), lowest in vascular plants (49-62Å) and intermediate in *Acetobacter* (70-84Å) (1). It appears that cellulose crystalline dimensions are independent of the type of terminal cellulose synthesizing complex. The idea that cellulose biosynthesis is not exclusively responsible for determining its crystalline dimensions has been proposed previously by Marx-Figini (52).

Raman spectroscopy and ^{13}C CP-MAS NMR techniques have proved important in the investigation of cellulose crystalline structure (3). Based on the nonequivalence of alternate β (1-4) glucan chain glycosidic linkages as determined by Raman spectroscopy, it was concluded that the basic repeating unit was a disaccharide (53). The slight right and left-handed deviations from a two-fold screw axis were approximated by those observed in the crystal structures of cellobiose and methyl-β-cellobioside model disaccharides. Cellulose computer models have also been generated based on the glycosidic oxygen bond rotational angles. These computer models were reported to better approximate the saddle position between the two major rotational angle energy of conversion minima (thought to be representative of native cellulose) than the cellobiose and methyl-β-cellobiose crystallographic models (54).

Microscopic evidence confirming cellulose I glucan chain polarity was reported previously with *Valonia* cellulose (55,56). Recently, parallel chain polarity also was demonstrated by the asymmetrical arrangement of silver-labeled reducing ends at only one end of *Acetobacter* cellulose I fibrils (57). Two distinct crystalline forms of cellulose I (I_α and I_β) were reported by Atalla and VanderHart (58), based on CP-MAS ^{13}C NMR evidence. Cellulose I_α and I_β differed only in their patterns of hydrogen bonding, while their molecular conformations were otherwise identical (3). All cellulose microfibrils were thought to be mixtures of I_α and I_β forms with the I_α form predominant in cellulose from siphonocladalean algae and *Acetobacter*, whereas vascular plant cellulose primarily consisted of the I_β form. These conclusions were recently modified (59) following observations of the preferential I_α susceptibility to acid hydrolysis and mechanical beating, as well as solid state ^{13}C NMR methods which enhance the crystalline core resonances. It was determined that vascular plant cellulose consists almost exclusively of the I_β form, with far less (if any) I_α content than reported earlier. The celluloses of *Mougeotia* and *Chara* were recently reported to be predominantly I_β (60). This evidence suggests that a correlation exists between the presence of solitary rosette/globule terminal cellulose synthesizing complexes and the assembly of I_β cellulose (60). Linear terminal complexes may be associated with the formation of a mixture of both I_α and I_β crystalline forms.

It now appears that cellulose I is not exclusively the native polymorph present in all organisms. The results reported originally by Sisson (61), which provided evidence that cellulose II was the native polymorph present in *Halicystis* (Ulvophyceae) cell walls, were recently reinvestigated and confirmed (62). Additionally, cellulose II producing mutants of *Acetobacter* have been isolated and analyzed with x-ray and low-dose electron diffraction (63). When cellotetraose is induced to crystallize in solution it forms a structure which has been used as a model compound approximating the crystallographic nature of cellulose II based on x-ray diffraction, electron diffraction and CP-MAS ^{13}C NMR evidence (64). Significantly, in all cases where *Acetobacter* cellulose synthase *in vitro* activity has been reported,

the product was cellulose II as determined by x-ray and low-dose electron diffraction (27,65). These observations indicate that cell-free synthesis of cellulose I is not known and that the spatial arrangement of components responsible for the biosynthesis of cellulose I may be easily disturbed. Therefore, the biosynthesis of cellulose II in nature may reflect alterations in the structures responsible for cellulose assembly in those organisms where it has been observed.

In Vitro Cellulose Synthase Activity

While partial purification of UDPG:1,4-β-D-glucan glucosyltransferase (cellulose synthase) from *Acetobacter* has been achieved (2,27,65), it has not been possible to demonstrate cellulose synthase activity in solubilized vascular plant membrane fractions. Instead, isolated vascular plant membranes produced β (1-3) glucan (callose) using UDPG as a substrate. Previously reported low levels of β (1-4) glucan *in vitro* synthesis in vascular plant solubilized membranes are now thought to represent xyloglucan biosynthesis (2,66). A possible candidate for the *Acetobacter* cellulose synthase has been purified as an 83 Kd concanavalin A-binding glycoprotein (65). An earlier report of *in vitro* cellulose biosynthesis by *Acetobacter* digitonin-solubilized membranes (67) is now considered to be in doubt since the product formed was reported as cellulose I and a correlation between the *in vitro* product formed and the observed electron diffraction pattern was not demonstrated (27). Cellulose synthase activity was localized on the plasma membrane of *Acetobacter* (27), and is known to be regulated by bis-(3′-5′)-cyclic diguanylic acid, which is degraded by a membrane-bound Ca^{2+}-sensitive phosphodiesterase (2,68). However, vascular plant cellulose synthase does not appear to be under similar control. According to Delmer (2), the vascular plant cellulose synthase is a multifunctional β(1-3):β(1-4) glucosyltransferase under the regulation of an 18 Kd 2,6-dichlorobenzonitrile (DCB) binding protein and Ca^{2+}. In order to test this hypothesis, antibodies raised against the DCB binding protein and callose synthase will be used to examine their affinity for rosette/globule terminal complexes (69). From the results of this research, it will be possible to determine whether cellulose synthase and callose synthase are the same enzyme complexes and if the terminal complex structure is actually associated with cellulose biosynthesis.

It also should be noted that Northcote (70; see chapter 1, this volume) has proposed a mechanism to explain the production of callose when vascular plant cells are damaged (including during membrane isolation for *in vitro* cellulose synthase activity). In this model, the cellulose synthase complex includes a binding protein which controls the orientation of the growing glucan chain non-reducing end. Under normal conditions, the C-4 hydroxyl at the non-reducing end is oriented so that it is the most favored site for transfer of the next glucose from UDPG. When cell damage occurs, the orientation of the binding protein is disrupted so that the C-3 hydroxyl is the most favored site for acceptance of glucose. However, this model fails to explain how the transfer of glucose to the glucan chain non-reducing end

is coordinated so that each successive glucose is rotated 180° relative to the adjacent one. No experimental evidence is currently available to determine the possible correlation between the Northcote model and electron microscopic observations of terminal complex structure.

Progress comparable to that made in understanding the β (1-4) glucan polymerization event has not been achieved with the cellulose crystallization process, since no evidence demonstrating the *in vitro* production of cellulose I has been reported. Haigler's cell-directed self-assembly model comes closest to explaining cellulose crystallization in *Acetobacter* (25,26). This model proposes that linear rows of outer membrane particle pores maintain nascent β (1-4) glucan chains in 1.5 nm nondissociable fibrils as they are extruded. Outside of the cell, glucan chains in register (established by the outer membrane pores) spontaneously self-assemble into 3.5 nm cellulose I microfibrils.

How the self-assembly of 1.5 nm fibrils occurs is the source of current debate. Ruben and Bokelman (71; see chapter, this volume) have observed 1.78 nm submicrofibrils arranged in a left-hand-twisted, triple-stranded pattern within 3.68 nm microfibrils by platinum-carbon shadowing for electron microscopy. They concluded that this type of construction was incompatible with the proposal (5,26) that microfibrils were formed by the lateral fasciation of 1.5-1.8 nm fibrils along crystal lattice planes. A helicoidal association of submicrofibrils mediated by the hydrogen-bond forces of xylose-containing hemicellulosic polysaccharides was suggested as the mechanism of self-assembly in *Acetobacter* (71). However, the recent evidence that crystal lattices of *Acetobacter* cellulose up to 25 nm wide were imaged by electron microscopy (72), suggests that continuous, uninterrupted crystalline domains of cellulose exist, which intrinsically follow crystal lattice planes. Furthermore, no evidence for the presence of xylose-containing polysaccharides in *Acetobacter* pellicles was confirmed (Gretz and Hotchkiss, unpublished data). Therefore, the self-assembly of 1.5 nm fibrils into microfibrils probably occurs in a helical fashion, but by a mechanism which maintains the symmetry of congruent lattice planes. More evidence is needed to prove that the self-assembly process is helicoidal.

Alteration of Cellulose Biosynthesis

Attempts to examine the process of cellulose crystallization have frequently involved culturing *Acetobacter* in the presence of fluorescent brighteners, direct dyes, carboxy-methyl-cellulose, or other agents which compete for interchain hydrogen bond sites, thereby disrupting microfibril formation (5,26). The sheet-like structure of the altered *Acetobacter* cellulose is now better understood following x-ray and electron diffraction analysis (73). The results of this study indicated that fluorescent brightening agents, such as Calcofluor, stack transverse to the glucan chain long axis and assume a helical orientation due to glucan chain twisting. The primary forces involved in the stacking of dyes to the nascent glucan chains were reported to be hydrophobic interactions. Helically twisted fibrils of cellulose I could be regenerated from the noncrystalline altered cellulose following water

washing. However, a reduction in crystallite size relative to that typical of *Acetobacter* cellulose was observed. Kai (74) reported that dye-altered cellulose was not totally amorphous, observing reflections at 6.0Å. In contrast, the only reflections Haigler and Chanzy (73) observed (3.99Å) with similar material were attributed to the helical stacking of the dye.

These results suggest that if the events of cell-directed extrusion and nascent glucan chain self-assembly become uncoupled (as in the case of cellulose regeneration from dye-altered cellulose), the native crystalline cellulose dimensions will not be achieved. The cell-directed self-assembly model is strengthened by this information, with the corollary that the cellulose synthase is localized on the plasma membrane as suggested by Bureau and Brown (27). It appears obvious that, regardless of how the nascent glucan chains traverse the peptidyl glycan cell wall and outer membrane, the critical structural components responsible for cell-directed extrusion are localized on the outer membrane. Absence of the linear row of outer membrane particles in cellulose II producing mutants (63) suggests that the cell-directed extrusion mechanism was altered, leading to the crystallization of cellulose II. Future research examining the self-assembly of extruded β (1-4) glucan chains in the presence of compounds that undergo cholesteric liquid crystal formation should also yield valuable information that will address the possibility of a helicoidal mechanism for microfibril construction.

Since the events of glucan chain polymerization and cellulose crystallization are not spatially separated in eukaryotic organisms as they are in prokaryotic organisms, the observation of cellulose II in the former (62,63) raises interesting questions concerning the structure of a terminal complex that could assemble antiparallel cellulose. One possibility is that the PF component contains the cellulose synthase activity and the EF component either is missing or lacks the ability to align nascent glucan chains in a parallel orientation. Alternatively, if cellulose II chain polarity is parallel instead of antiparallel, terminal complex mediated cellulose II assembly would be much more easily explained based on our present knowledge. A parallel crystal structure model for cellulose II has been described recently (75).

It is interesting to consider the effects of non-cellulosic cell wall polysaccharides on cellulose crystallization in eukaryotic organisms. The addition of purified pea xyloglucan (76) or mannodextrins (Atalla, personal communication) to *Acetobacter* cultures has been reported to prevent or alter cellulose microfibril crystallization. These results suggest that plant cell wall polysaccharides present during microfibril deposition may alter cellulose biosynthesis. Based on ^{1}H-NMR evidence (second moment and solid echo analysis), a model was proposed which suggests that cellulose microfibrils form a highly ordered complex with an immobile-population of xyloglucan in the primary cell walls of bean hypocotyls (77). While this model is preliminary, it appears to imply that the intimate association with xyloglucan may be due to crystallization of cellulose in the presence of xyloglucan at the plasma membrane. These proposals represent a possible explanation for the observed variation in cellulose crystallite sizes that would be open to developmental regulation based on changes in cell wall composition.

Cell wall components also have been reported to influence the pattern of microfibril deposition. Glucuronoxylan has been affinity-labeled (xylanase-gold complex) preferentially at the points of helicoidal microfibril orientation shifts between S_1 and S_2 lamellae in *Tilia platyphyllos* wood (78). It was proposed that the structural attributes of the hemicellulosic glucuronoxylan (elongated stiff backbone with short, flexible side chains) were favorable for cholesteric liquid crystal formation. Therefore, through close association with the cellulose, the glucuronoxylan could induce a helicoidal transition between S_1 and S_2 microfibril orientations. The turnover of xyloglucan in dicots and β-glucan (mixed 1-3, 1-4 linkages) in monocots catalyzed by specific cell wall localized glucanohydrolyases in addition to Ca^{2+}/H^+ ion exchange by acidic cell wall carbohydrates, are thought to allow the slippage reorientation of cellulose microfibrils to occur during cell elongation (79). Cell wall glycoproteins may also influence the spatial arrangement of cellulose microfibrils. The distribution of isodityrosine cross-links between adjacent hydroxyproline-rich glycoproteins has been proposed to establish a cell wall matrix mesh which restricts the deposition of cellulose microfibrils in vascular plants (80).

Molecular Genetics of Cellulose Biosynthesis

Biochemical knowledge of biosynthetic metabolism has often been aided by genetic studies of mutants defective in key enzymes in various anabolic pathways. It is hoped that this research approach will also be helpful in investigations of cellulose biosynthesis. Currently *Acetobacter* mutants deficient in cellulosic pellicle production (Pel^-) have been produced (81,82). However, the Pel^- mutants produced in one study (82) still made small quantities of cellulose II *in vivo*, possessed normal UDPG:1,4-β-D-glucan glucosyltransferase activity *in vitro* and had no detectable galactose in lipopolysaccharides (LPS). These observations were interpreted to mean that the Pel^- defect was not in the cellulose polymerization event but that the defect may have been in the preceding step catalyzed by UDPG pyrophosphorylase (83). However, an additional mutation affecting the structures responsible for nascent glucan chain translocation through the peptidyl glucan and outer membrane may also be present since cellulose II is produced *in vivo*. The alteration in lipopolysaccharide structure may be a manifestation of the latter type of defect in Pel^- mutants. Further investigation of these mutants should provide valuable information about the mechanism of cellulose II formation and glucan chain translocation in *Acetobacter*.

Isolation and sequencing of the cellulose synthase gene(s) has not been accomplished yet; however, DNA from *Acetobacter xylinum* containing this gene(s) was cloned into broad host-range plasmid vectors (82). These vectors were mobilized into Pel^- mutants to test for complementation. To date, this approach has not produced a pellicle-forming transconjugant from a Pel^- mutant of *Acetobacter* (82). The direct correlation between cellulose production and presence of plasmid DNA in *Acetobacter* has been reported

(84), suggesting that the cellulose synthase gene(s) was localized on one or more plasmids. However, some *Acetobacter* strains lacking plasmids or cured of plasmids were recently reported to produce cellulose (82). Therefore, plasmids cannot be regarded as the exclusive site for cellulose synthase genes.

Conclusions

Much progress has been made in the field of cellulose biosynthesis in the past few years. The distribution of terminal complexes in plants has been more fully described. The gap between ultrastructural observations of terminal complexes and biochemical evidence for their function in cellulose biosynthesis has been narrowed, leading to a growing acceptance of the terminal complex hypothesis in the scientific community. In *Acetobacter*, the cellulose synthase has been localized on the plasma membrane and significant progress has been made toward its isolation. High resolution evidence has been presented to describe the process of cell-directed self-assembly of *Acetobacter* cellulose ribbons. Future examination of the role of cholesteric liquid crystallization in cell-directed self-assembly may help to resolve differences between this model and the triple-stranded, left-hand-twisted cellulose microfibril model for cellulose crystallization. The diversity of cellulose physical structure in nature has been further defined. Especially significant in this regard have been the observations of the correlation of terminal complex type with cellulose I_α and I_β structure and the occurrence of native cellulose II.

Due to the abundance of literature concerning cellulose structure and biogenesis, this review was not intended to be comprehensive in nature. Instead, interpretation, speculation and analysis of recent progress in various areas of cellulose biosynthesis research have been offered in an attempt to stimulate new ideas and discussion. Many of the recent investigations enumerated can potentially make significant contributions toward a better understanding of cellulose structure and its biosynthesis in the future. The author agrees with Delmer (2,85) that there is ample opportunity for new contributors and novel approaches in this enigmatic field.

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